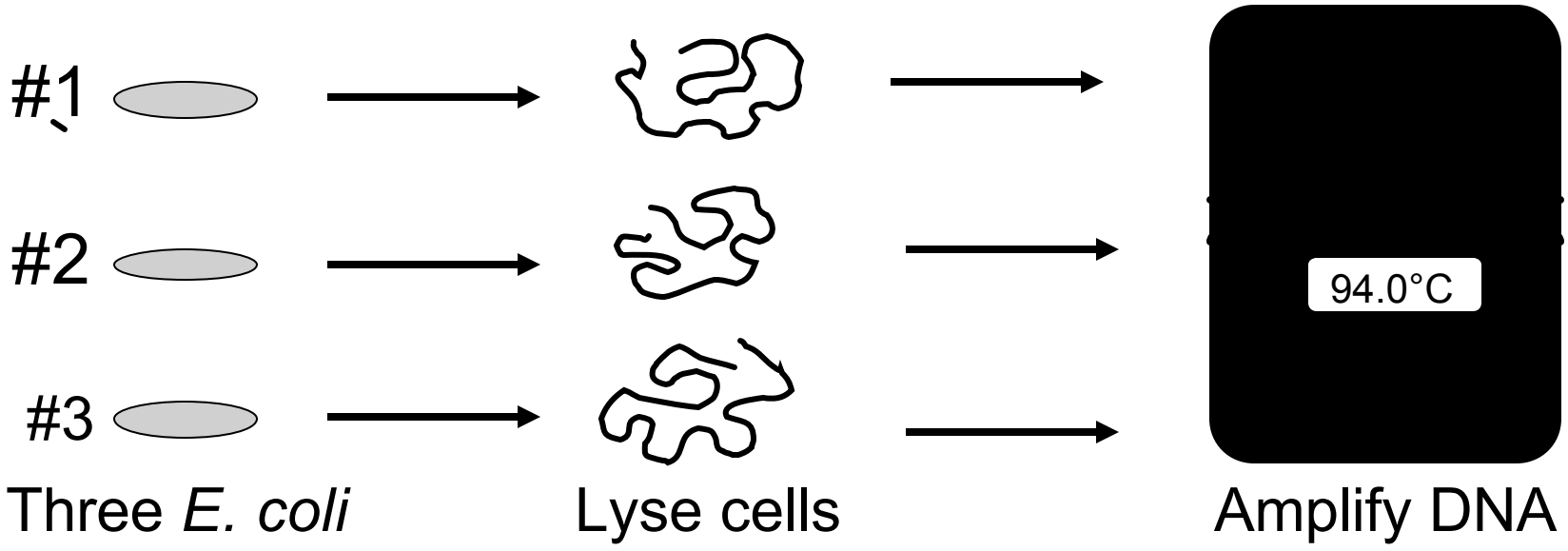
Repetitive Extragenic Palindromic (rep-PCR)

Highly-conserved repeated DNA elements

~ 500 copies of the REP consensus sequence in the E. coli chromosome.

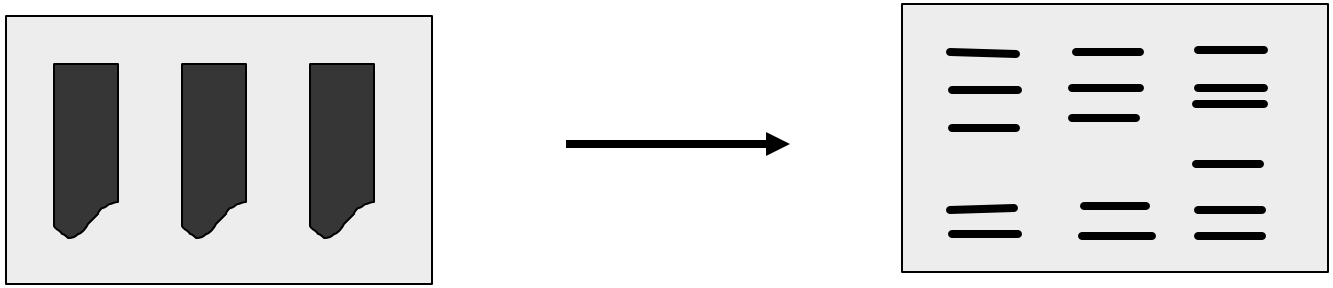
rep-PCR



Three *E. coli* isolates from environment

Lyse cells

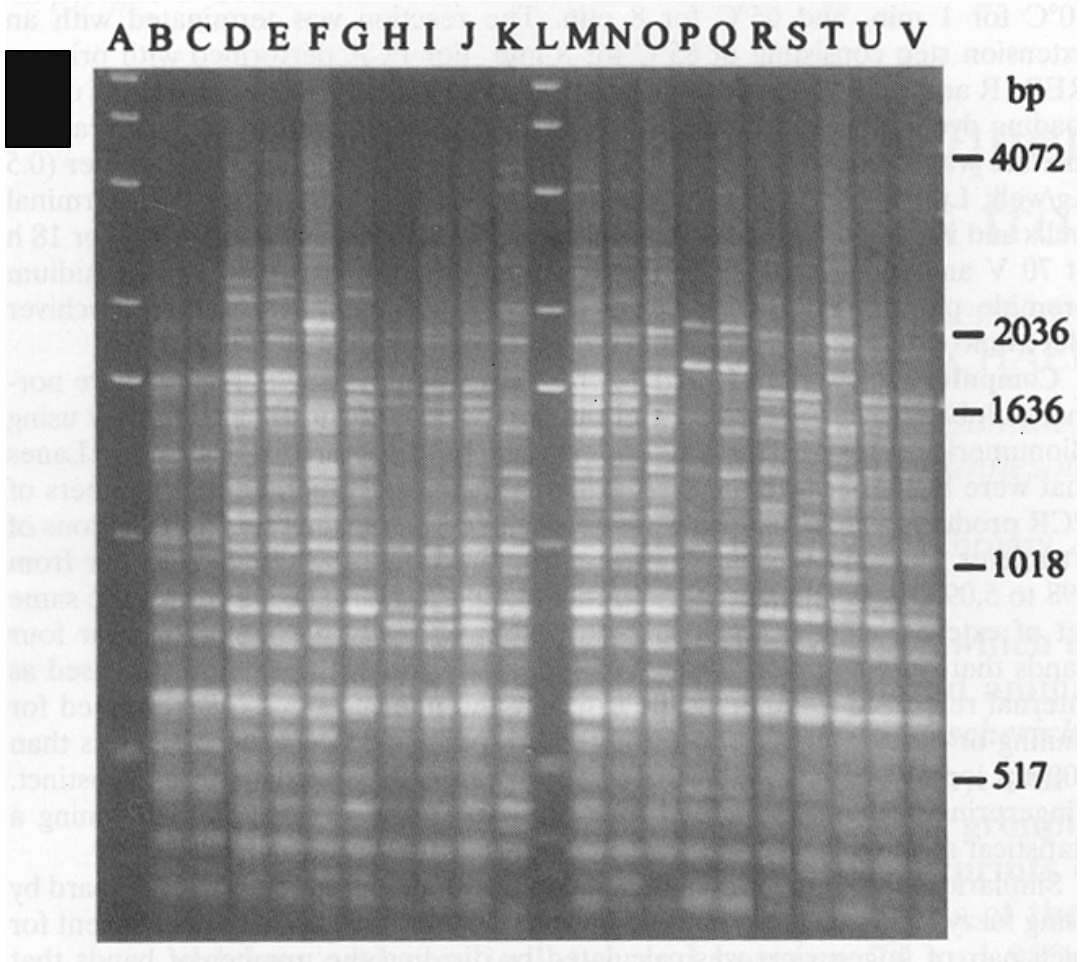
Amplify DNA



Run DNA on gel

Stain DNA to give "fingerprint"

rep-PCR DNA Fingerprint



Adapted from Dombek et al. (2000)

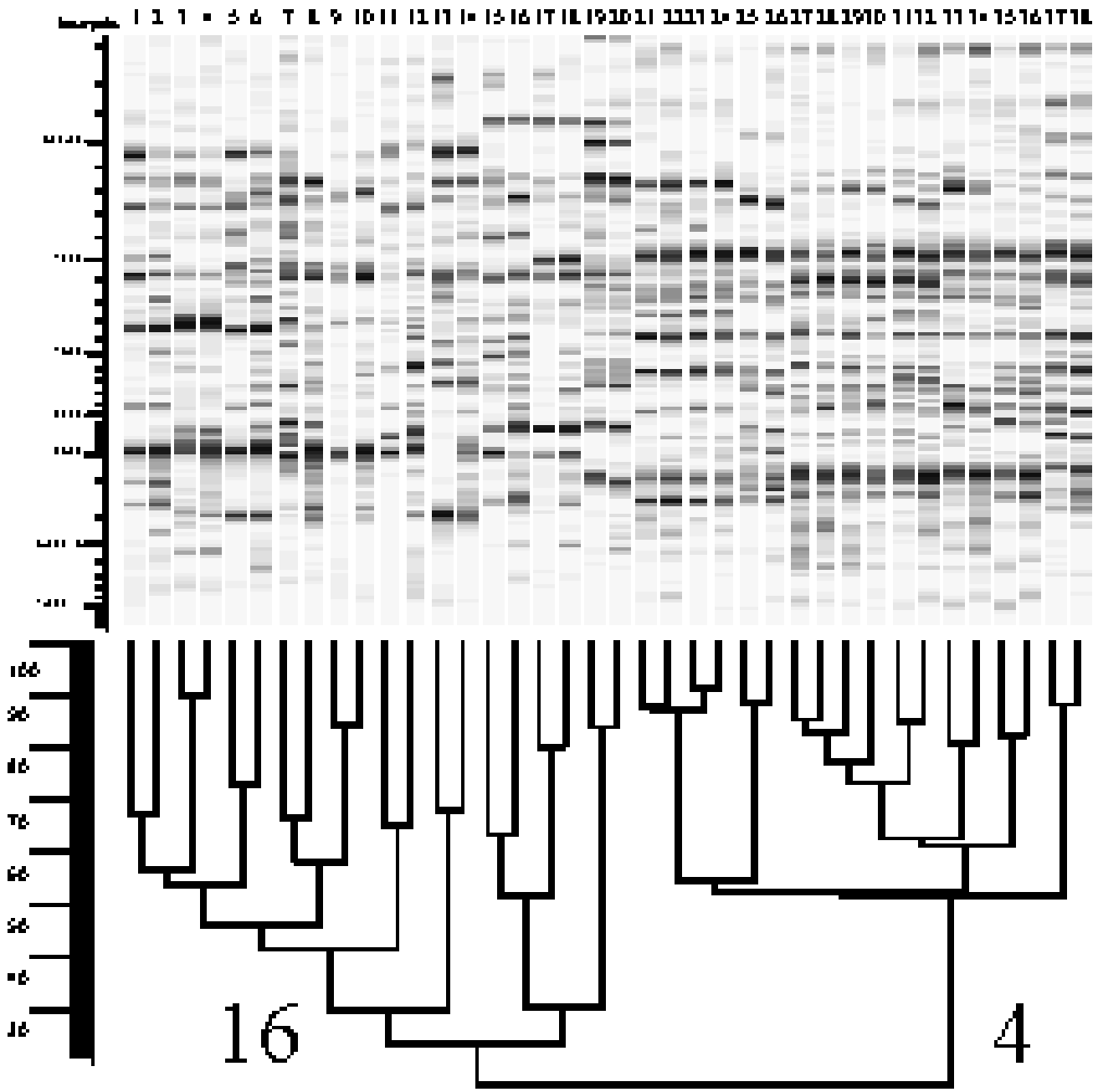


Fig. 7. Cluster analysis of REP-PCR genomic fingerprints of 38 (non specified) *Xanthomonas* strains belonging to DNA homology groups 4 and 16 (Vauterin *et al.* 1995) using the product moment and UPGMA method.

rep-PCR

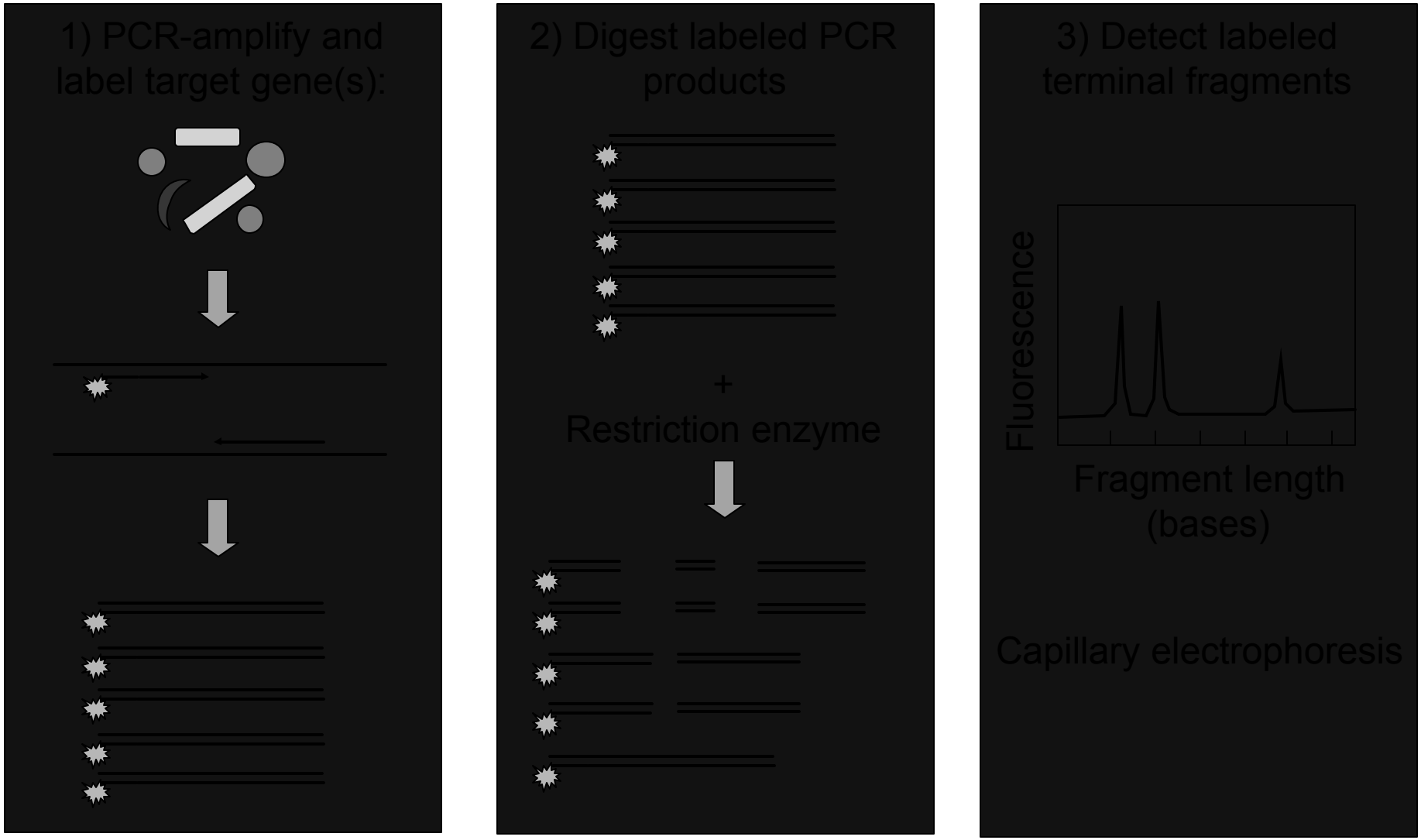
Advantages

- Highly reproducible
- Excellent discrimination

Disadvantages

- Knowledge of DNA sequence needed
- Amplifying errors

T-RFLP (terminal restriction fragment polymorphism)



PCR = polymerase chain reaction

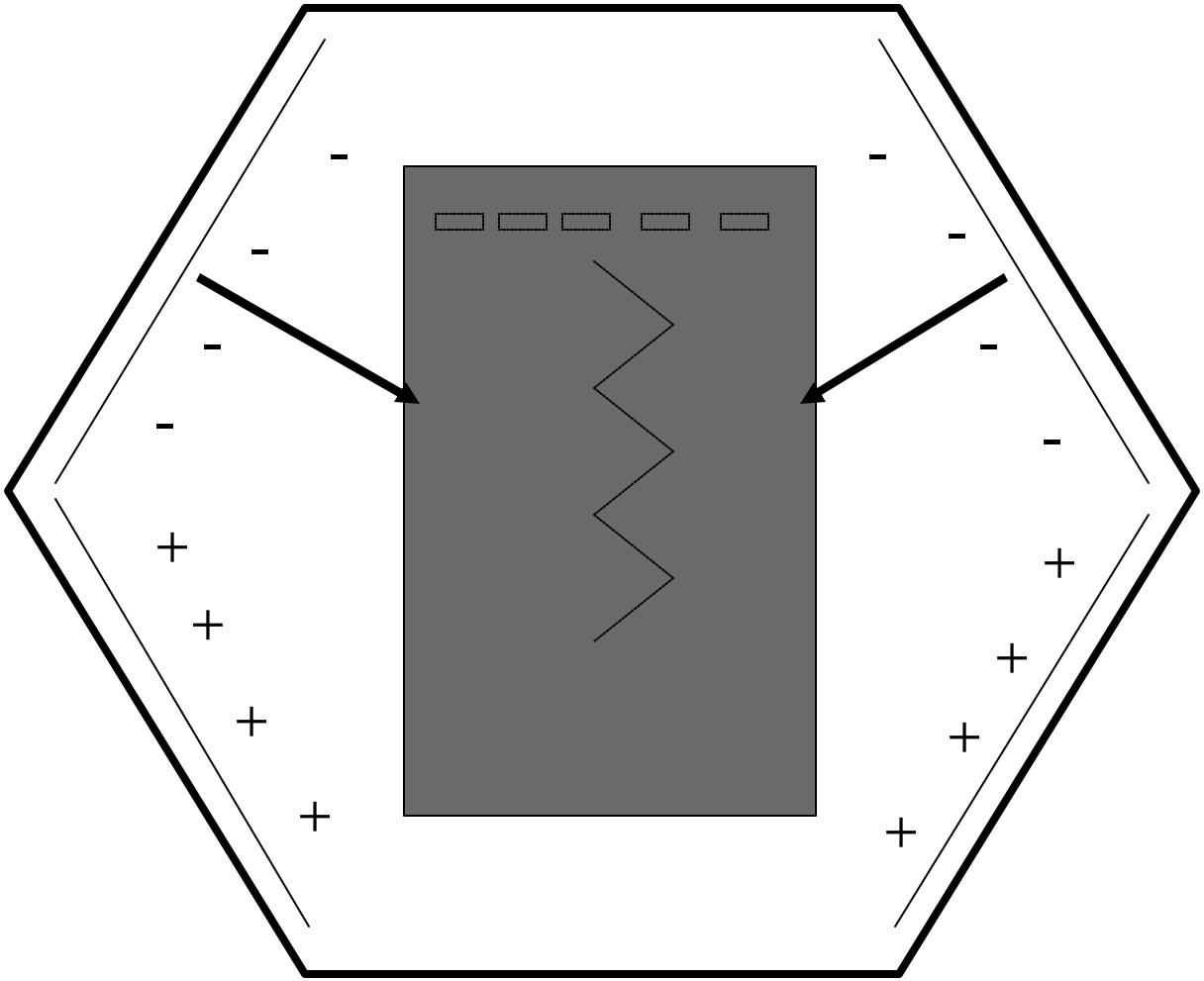
Terminal Restriction Fragment Length Polymorphism (T-RFLP)

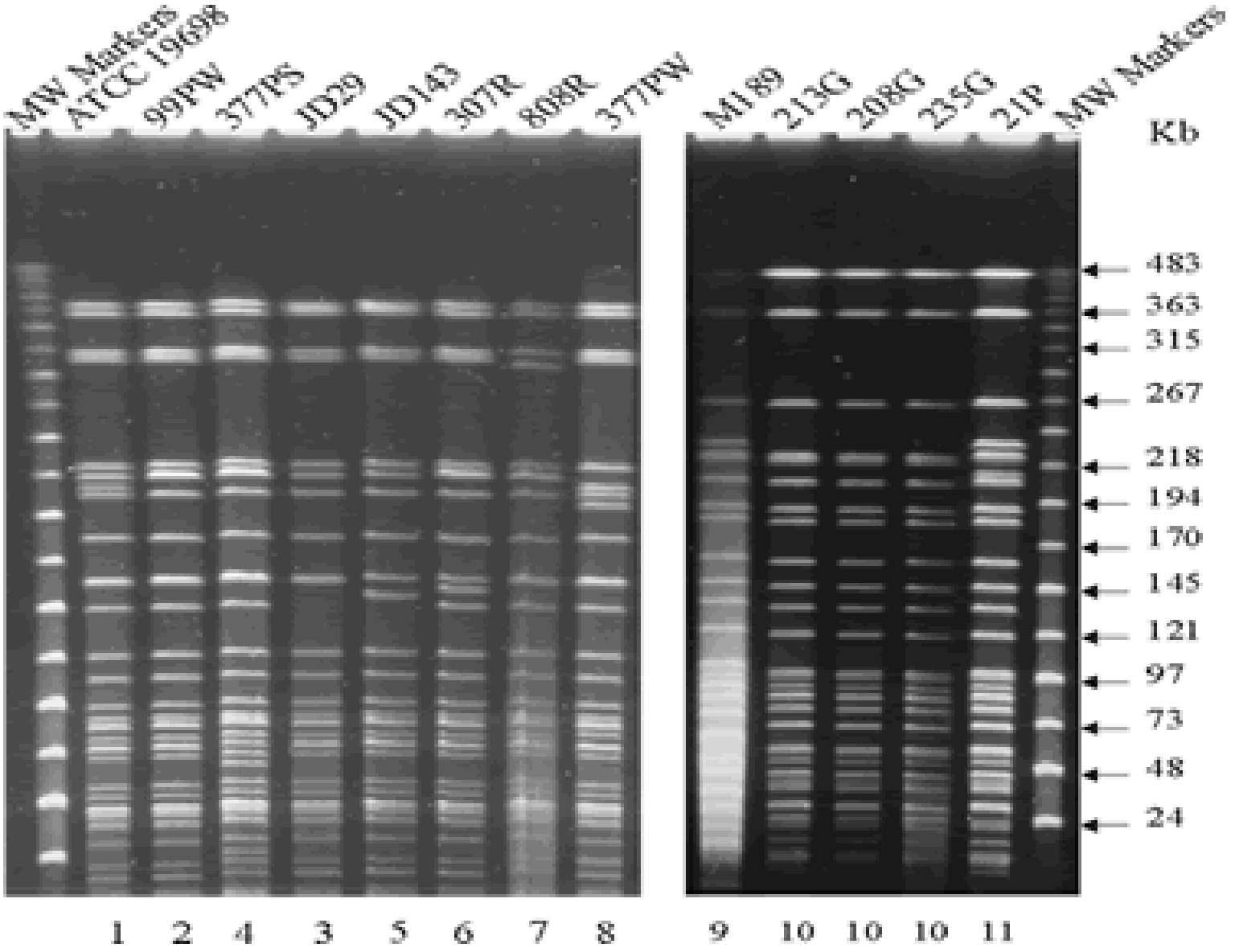
Advantages	Disadvantages
Does not require culturing	Expensive equipment needed
No library needed	Technically demanding

Pulse Field Gel Electrophoresis

Switch Time

Electric Field 1 ---------- Electric Field 2




MW Markers
ATCC 19698
99PW
377PS
JD29
JD143
307R
808R
377PW
M189
213G
208G
235G
21P
MW Markers
Kb
483
363
315
267
218
194
170
145
121
97
73
48
24
1
2
4
3
5
6
7
8
9
10
10
10
11

Pulse Field Electrophoresis (PFGE)

Advantages

Used in genotyping and epidemiology

High discrimination

Reproducible

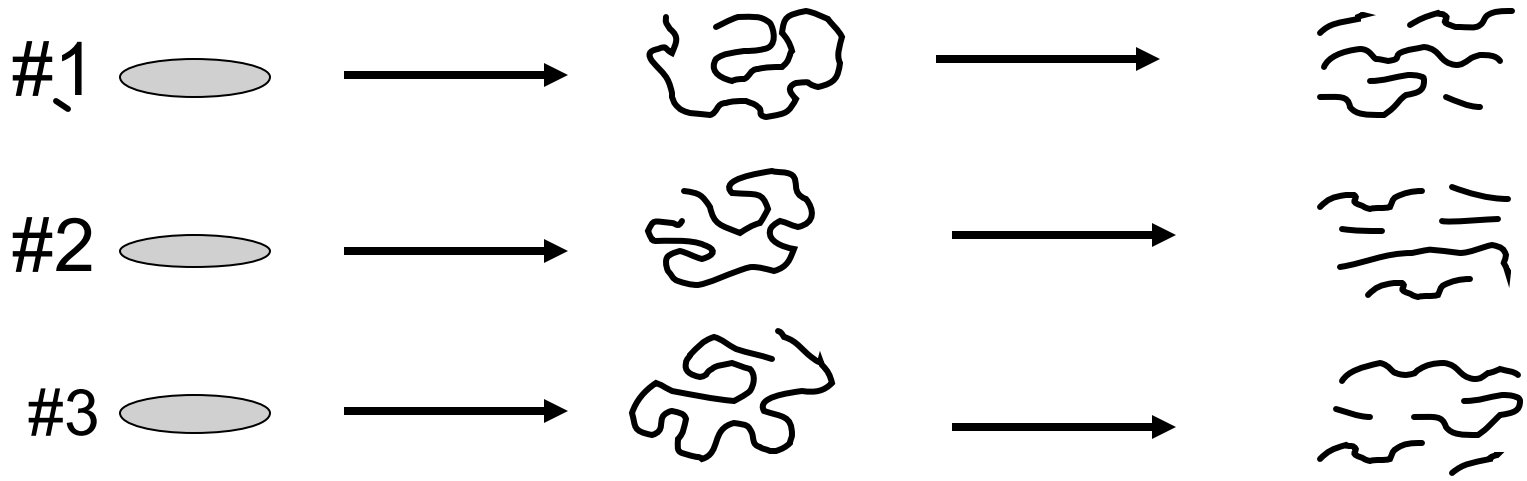
Conclusive results

Disadvantages

Long assay time

Limited Simultaneous strains processing

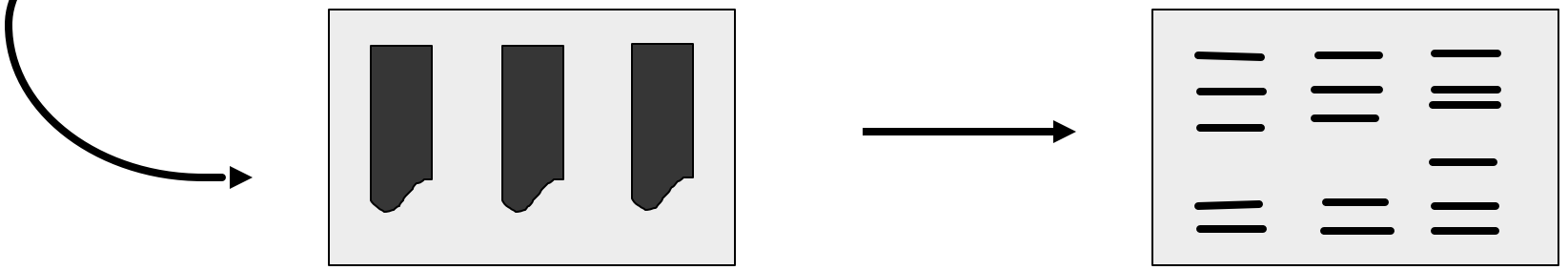
Ribotyping



Three *E. coli* isolates from environment

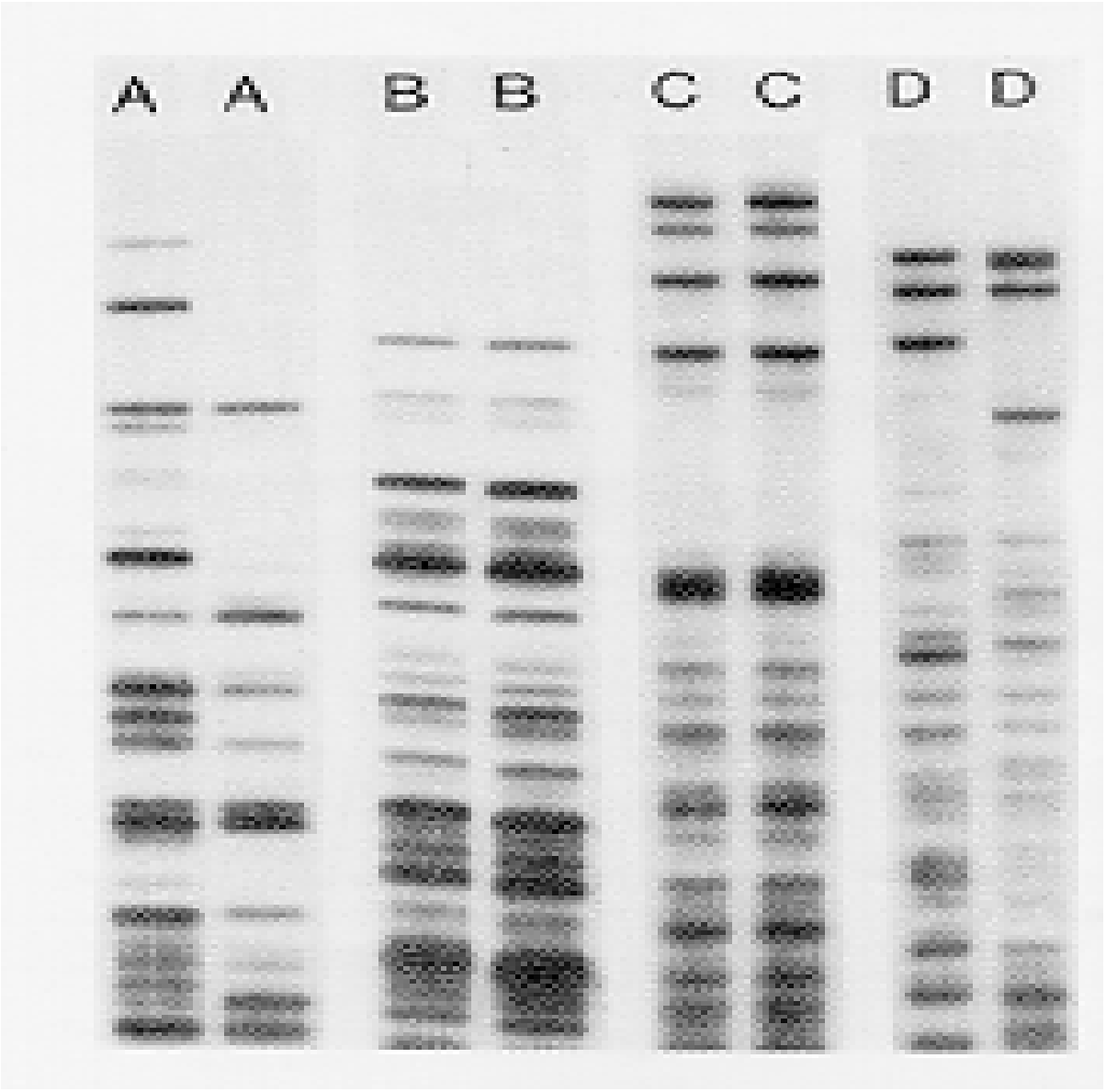
Isolate DNA

Cut DNA



Run DNA on gel

Probe membrane with labeled DNA to give “fingerprint”


A
A
B
B
C
C
D
D

Ribotyping

Advantages

- Easy to type
- Highly reproducible
- Easy to perform
- Easy to interpret
- Easy to automate

Disadvantages

- Tedious
- Time-consuming
- Expensive

MST Methods
Perception vs. Reality

- Great interest and need for a reliable molecular method for MST.
- Many methods being studied but none fulfills the need very well. Not ready for primetime!
- All potential MST molecular methods require well trained analysts, are technically challenging and are expensive.
- MST research needs are nearly overwhelming.

MST Methods Research Needs

- Host origin database
- Biogeographic variability
- Temporal variability
- Environmental factors (e.g., rainfall)
- Indicator organisms
- Automation